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PREPARATION AND TESTING OF RADIOVACCINES

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After Ashkinass and Kaspari(1) demonstrated the bactericidal power of ionizing radiations in 1901, a long series of studies have made it possible to prove that the radiosterilizing mechanism greatly differs from that of regular sterilizing agents such as heat and antiseptics.

According to these studies, the action of ionizing radiations upon live cells seems, in fact, to be much finer and more sophisticated and, to put it in Lacassagne's(2) words, the irradiation constitutes "a sort of microdissection leading to a dissociation of the cellular functions". The first visible property is the ability to multiply which is considered one of the main factors of virulence. By using appropriate absorbed doses, it is possible to destroy this power in a bacterial population. Unable to continue proliferating in the regular environment, the population finds itself completely and definitively sterilized in a bacteriological sense of the word.

We have shown(3), however, that cultures thus sterilized, while deprived of their virulence vis-a-vis test animals, continue to possess most of the properties of live bacteria and in particular their mobility, respiration, and antigenic powers(4,5). Radiosterilization appears to be a particularly interesting process for preparing vaccines whose immunizing power must be very close to that of the live germs. (The latter constitute, in principle, an efficient vaccine once their virulence has been suppressed.)

This dissociation of cellular functions seems to be a general property of ionizing radiations and we have found it to be true for very different types of radiations: alpha rays (radon), X-rays (210 kV), gamma rays (^{60}Co), total radiation of a reactor (gamma and neutrons), boron fission rays and 150 MeV protons.

The target theory, which attributes to each cellular function a characteristic effective section vis-a-vis radiation, affords a fairly satisfactory interpretation of the facts observed.

Qualitative differences between sterilization by irradiation and sterilization by heat or antiseptics, also appear on a quantitative level. Radiosterilization generally takes place according to an exponential law, i.e., the number N of germs surviving a dose D is given by the formula

$$N/N_0 = e^{-kD} \text{ and } \text{Log } N = (\text{Log } N_0 - kD) \quad \begin{array}{l} N_0 = \text{number of germs} \\ \text{for } D = 0 \\ k = \text{proportional factor} \end{array}$$

This exponential is only an individual example of the general law of Poisson utilized in the target theory, and in certain cases more complex laws corresponding to Poisson curves of an order above 1 can be observed. Radiosterilization is not an "all or nothing" phenomenon which takes place beginning with a certain dose, as heat sterilization is above certain temperatures or chemical sterilization for certain concentrations. An absorbed dose in a bacterial suspension corresponds to a reduction of surviving germs, i.e., to a sterilization probability "which leads us to a notion of relative, statistical sterilization, a bit out of tune with our old, deeply-rooted concepts" (7). Each microorganism has a characteristic radiosensitivity which nevertheless varies according to the medium where it is irradiated and the type of irradiation utilized. If inactivation is exponential, this radiosensitivity is generally expressed by dose D_{10} reducing the bacteria concentration by a factor of 10, i.e., leaving 10% alive; this D_{10} dose varies with the nature of the medium, which may afford a certain degree of protection against radiation.

In preparing vaccines, it is necessary to accurately determine the minimum sterilizing dose (MSD) insuring a regular sterilization of a bacterial suspension with a safety coefficient of the same order as that for heat or chemical sterilization. In order to keep the immunizing power of radioinactive germs intact, it is best to utilize the exact dose needed to insure a regular sterilization of the suspension. In practice, a safety margin of 2 or 3 D_{10} has appeared to be generally satisfactory, so that the sterilization dose of a medium containing 10^9 germs/cm³ will be approximately $9 + 3 = 12 D_{10}$; for example, the radiosterilization by gamma rays of a 10^9 germ/cm³ suspension of S. Typhi in physiological serum ($D_{10} = 10,000$ rad) will require approximately 0.12 Mrad.

We have studied the radiosensitivity with respect to the various radiations of a certain number of pathogenic germs, and after determining the minimal sterilization dose (DSM) we have prepared radiovaccines whose immunizing effects have been compared by some to those of the corresponding thermovaccines.

1. VIRUS

The antigenic properties and structure of viruses are very different from those of bacteria and much less favorable to the disassociation of virulence and antigenic power by ionizing radiations.

However, research on the irradiation of viruses with a view to determining their size and structure by the ultra-micrometry statistical method(5) has led us to radiosterilize certain pathogenic viruses and we have taken advantage of the occasion to test their protective power on the target animal.

Irradiation has been practiced on pulverized cells in normal salt water and the titre, determined on the animal by using statistical methods(6), was usually on the order of 10^5 to 10^6 virions/cm³.

1.1. Vaccine and anphthous fever viruses

Inactivation curves (fig. 1 and 2) show that the DSM for the anphthous fever dermatoph (O. Vallee strain) is 5 Mrad for alpha particles (of Rn) and 7 Mrad for X-rays (200 keV). For the vaccine, the DSM is 0.5 Mrad in alpha particles and 12 Mrad in X-rays. The immunizing power for these two viruses on the target animal appears to be practically nil.

1.2. Poliomyelitis virus

The possibility of obtaining by irradiation a vaccine retaining an antigenic power close to that of the live virus led us to study the radiosensitivity of this virus for various ionizing radiations.

A series of experiments with the total radiation of the first French ZOE reactor allowed us to determine the dose-effect curve for this radiation composed of neutrons and gamma rays (fig. 3). Sterilization of a culture medium was obtained with a particular flow of $2.5 \cdot 10^{16}$ n/cm², or approximately 25 Mrad of total radiation.

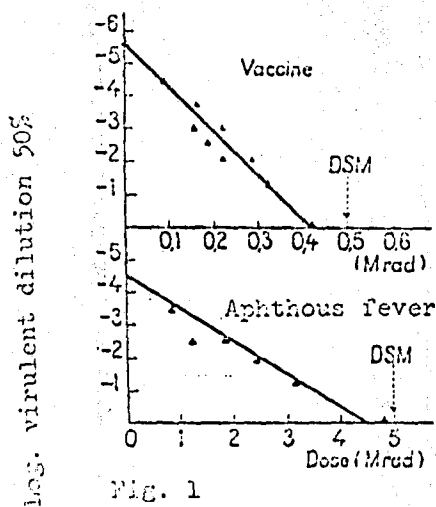


FIG. 1
Irradiation of vaccine and anphthous fever viruses with radon alpha particles,

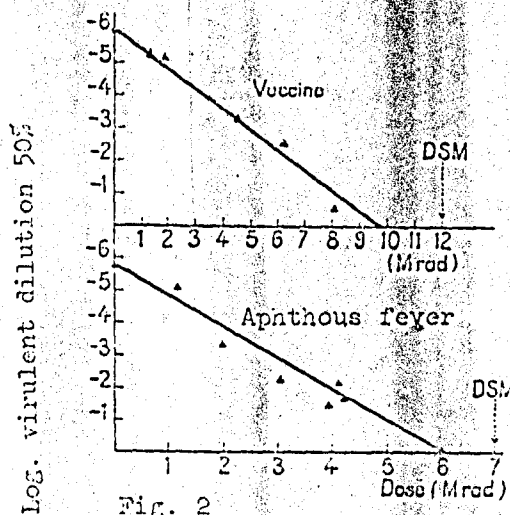


Fig. 2
Irradiation of vaccine and anphthous fever viruses with X-rays.

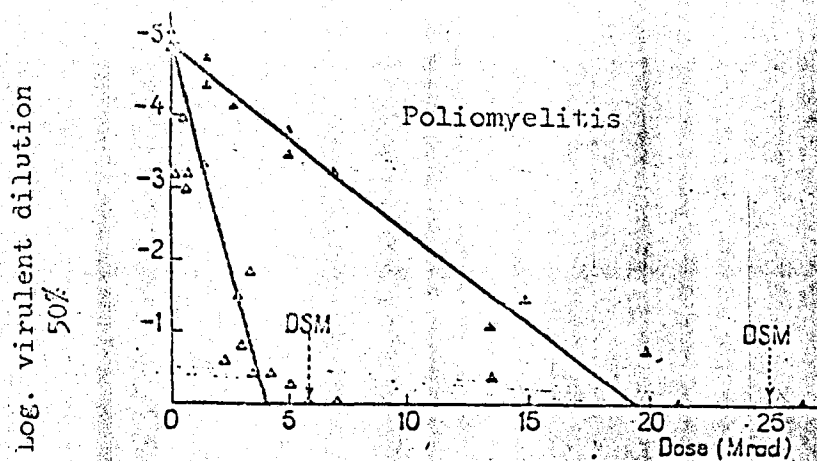


Fig. 3. Irradiation of poliomyelitis virus with total radiation of the ZOE reactor.

To reduce this dose, the virulent suspension has been supplemented with 5% boron (sodium borate); the presence of the boron, while not changing the titre of the virus, increases the efficacy of the radiation, since the fast-moving neutrons cause the boron split, resulting in a high production of linear energy transfer radiations (TLE). The DSM is thus reduced to 6 Mrad, or by a factor of 4 (fig. 3).

While the total radiation of a reactor makes it possible to sterilize large quantities of vaccines, with a high TLE radiation, it has the disadvantage of producing artificial radioelements in the vaccine, some of which have a high radiotoxicity. We repeated these experiments with ^{60}Co gamma rays which do not present this disadvantage and determined the radioinactivation curve (fig. 4); a DSM of 4 Mrad appeared to be satisfactory for the preparation of a radiovaccine.

An initial immunization test on animals using this radiovaccine revealed that virulence was completely eliminated, but that the antigenic properties were very weak.¹

2. BACTERIA

To obtain injectable vaccines, bacterial suspensions were prepared in a normal saline solution, using a concentration on the order of 10^9 germs/cm³. Irradiation was first effected using alpha particles (Rn), and subsequently, using ^{60}Co gamma rays from an 800 Ci irradiator(6).

1. The details of these biological and immunization tests made with poliomyelitis virus subjected to various doses of neutrons and gamma rays is related by P. Lépine in a report addressed on 11 Dec 1962 to the International Atomic Energy Commission in Vienna, in fulfillment of research contract No. 17.

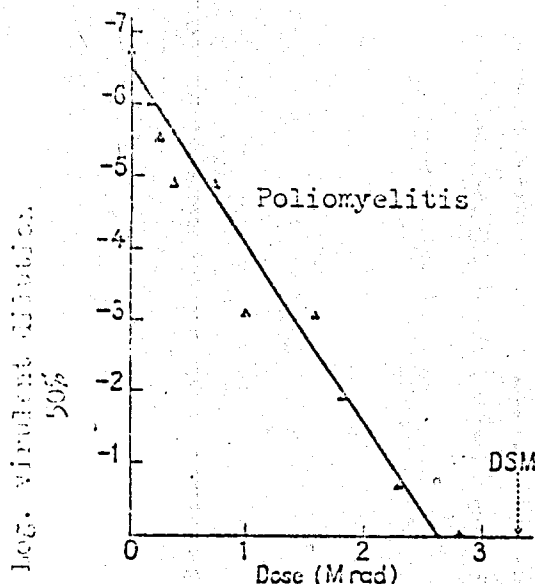


Fig. 4. Irradiation of poliomyelitis virus with ^{60}Co gamma rays.

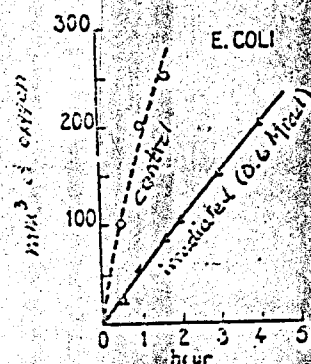


Fig. 5. Respiration of E. coli sterilized by X-rays.

2.1. Escherichia coli

For a pathogenic strain, using mice, the D10 dose is 7 Mrad of alpha particles. Sterility of a culture medium is usually obtained with 50 Mrad.

Virulence in the mice is completely eliminated, but the radiosterilized bacilli retain their mobility in the same manner that live bacilli do, and their respiration, measured with a Warburg respirometer, continues to show values slightly below those of the controls (Fig. 5). After a dose of 0.6 Mrad, or 10 DSM of X-rays, respiration still remains at a quarter of that of the controls (4). An identical heat-sterilized suspension shows neither mobility nor respiratory exchanges.

A test injection of 10^9 live bacilli supplemented with mucin triggers a mortality rate of 67% among non-vaccinated controls. Among the vaccinated, the mortality rate is 43% for thermovaccine and 22% for radiovaccine(5).

Although the reduced virulence of E. coli makes it unsuitable for a comparative study of immunizing power, the radiovaccine shows very clearly certain special properties--retention of mobility and respiration, and an immunizing power equal to or greater than that of thermovaccine.

2.2. Koch bacillus

The efficacy of BCG, an avirulent live vaccine, made us believe(9) that a radiovaccine prepared from a virulent BK strain might have comparable properties.

Irradiation by alpha particles of a normal saline solution suspension containing 1 mg/cm^3 of fresh bacilli reveals that 48,000 rad are necessary to completely eliminate the power of proliferation in a Löwenstein medium. But to destroy virulence in guinea pigs, the sterilization dose must be increased to 85,000 rad. This radiosensitivity corresponds to a D10 of 8,000 rad, on the order of that of bacteria of the same size, and the BK does not appear to show any special resistance to the ionizing radiations.

We prepared, using 0.1 Mrad alpha particles, a radiovaccine based on a virulent strain isolated of tuberculosis. This vaccine proved, as a general rule, to be void of any virulence whatsoever for the guinea pig. We verified, using the Warburg respirometer, a remarkable persistence in the respiration of the radiosterilized bacilli, for the latter showed a respiration equal to 20% of that of the controls for 4 days, while the respiration for the heat-sterilized bacilli was nil.

The immunizing power was tested on guinea pigs and a vaccination test resulted in a survival rate of 133 days \pm 30 among the controls and of 250 days \pm 40 among the radiovaccinated subjects.

2.3. Plague bacillus and Malassez and Vignal bacillus.

We studied the radiosensitivity of the plague bacillus, as well as that of the Malassez and Vignal bacillus. The latter is avirulent, has a dose resemblance to the plague bacillus, and appears to lend itself to classification in the same genus, Yersinia, of which it would be a species(10).

Irradiation with alpha particles of the Malassez and Vignal bacillus (strain 5-I) reveals a regular elimination of proliferation in peptonized water for doses greater than 0.2 Mrad.

The plague bacillus (virulent strain H III-50) shows a radiosensitivity of the same order, with a slightly smaller DSM of 0.18 Mrad.

2.4. Vibrio cholerae

Experiments have been carried out on Ogawa and Inaba strains coming from the National Institute of Health (Bethesda). For these two strains, a dose of 0.15 Mrad of alpha particles overcomes the reproductive power in a culture medium, while mobility is clearly restrained.

With a normal saline suspension containing $2 \cdot 10^9$ vibrios Inaba per cm^3 and the same number of vibrios Ogawa, a thermovaccine (two heatings of one hour at 56°C) and a radiovaccine (0.15 Mrad) was prepared. The toxicity test on mice showed the same innocuity for the vaccines.

A comparison of the immunizing effects was made as follows:

Four groups of 22 mice received in intra-peritoneal injection of vaccine containing, in the same 0.5 cm^3 volume, $3.2 \cdot 10^5$, $1.6 \cdot 10^6$, $8 \cdot 10^6$ and $4 \cdot 10^7$ vibrions. Fourteen days after the vaccination, the mice received a test injection of 1,000 DL50, either of the Inaba vibriion or of the Ogawa vibriion.

As mortality among the mice during the immunization period surpassed the permissible maximum, the DL50 was not determined by the Wilson and Worcester method. Rather, the log-probit(10) graph method was used. The immunizing power of the two vaccines was shown to be practically identical.

2.5. Salmonella typhi

We used principally(11) the TY2 (form V) strain; an 18-hour culture is put into suspension, in various mediums, with a concentration of 10^9 germs/ cm^3 .

The DSM varies with the medium. For alpha particles (and 150 MeV protons), it is 90 Mrad (fig. 6) in normal saline solution, but in bouillon it is 0.5 Mrad. In a 7.5% glucose medium (chosen in view of the dry irradiation of the lyophilized germ)(12), the DSM is 0.25 Mrad. Similar results are produced by using a 15% lactose medium, 1.25% saccharose, and a 1.25% sodium glutamate medium.

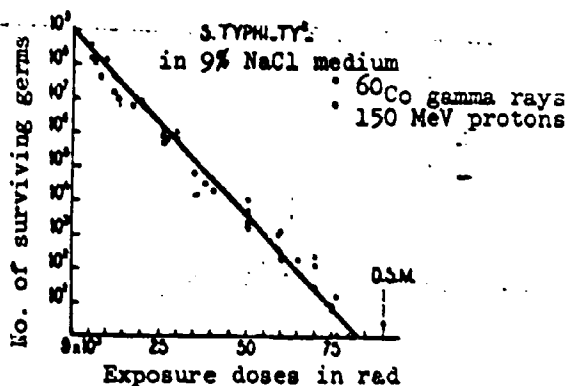


Fig. 6. Irradiation of S. typhi with gamma rays and 150 MeV protons.

The germs remain completely mobile, even at doses which are greatly in excess of the sterilizing dose; at 2 Mrad, mobility appears to be even greater than that of the controls(13), while the heated bacteria remain immobile, taking Brownian movement into consideration.

The Warburg respirometer again shows the continuation of respiration in proliferating and non-proliferating media for cultures radiosterilized at 0.6 Mrad (fig. 7). The disappearance of the power to reproduce was controlled at several moments during the experiment.

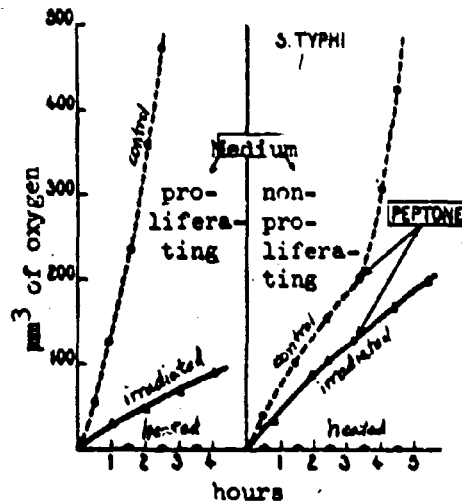


Fig. 7. Respiration of *S. typhi* sterilized by gamma rays (0.6 Mrad).

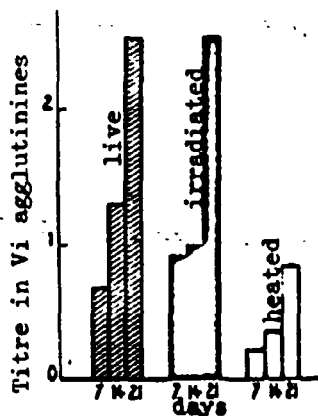


Fig. 8. Production of antibodies in rabbits. Average titre of serum in Vi agglutinines.

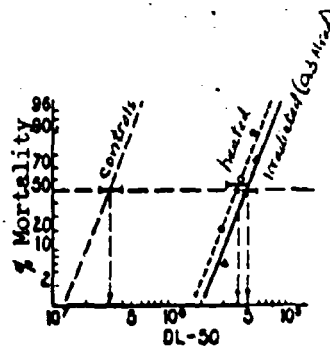


Fig. 9. Comparison of immunizing power, for mice, of heated and irradiated vaccine in a 7.5% glucose medium.

For all the strains studied (forms V and W), the ionizing radiations have always respected the serological integrity of the antigens O, H, and Vi(14).

The production of antibodies among rabbits was studied and Figure 8 shows that the radiovaccine produces an array of antibodies (O,H,Vi) completely comparable to that obtained with live bacteria and superior to that from thermovaccines for Vi antibodies.

The immunizing power was determined on mice; it is given by the immunity index $R = \text{ratio of DL50's of new and vaccinated animals}$ (15). The vaccination was effected by means of two subcutaneous injections of 10^8 germs (heated or irradiated) at 7-day intervals; 14 days after the second injection, DL50 of the ty2 test strain was intraperitoneally administered to the controls and the two vaccinated groups at the same time. A comparison of the protective power of the vaccines was effected by determining the R_p ratio of the immunity indices R_1 and R_2 of the two vaccines and comparing it to the R_p -factor of the reliability limits of this ratio; if R_p is greater than R_p , the difference at the 5% level(16).

The results shown in fig. 9 reveal that the two vaccines have a fairly comparable immunizing power.

The radiosterilization of B. typhi, then, leaves the germ very close to the live bacillus (mobility, respiration). Once avirulent, the bacillus retains these antigenic properties and its immunizing power is at least equal to that of heated vaccines (by the active protection test on mice). Experiments are underway on the radiosterilization of the lyophilized germ.

CONCLUSION

The radiosterilization of the viruses studied is obtained using doses of alpha particles on the order of 3 to 10 Mrad with the exception of the vaccine; the radiovaccines of these viruses have not shown any notable protective power.

The bacterial radiovaccines prepared in normal saline solution using doses comprised between 50 and 200 Mrad retain certain properties of the live bacilli (mobility, respiration, antigenic power) and show a protective power at least equal to that of the heated vaccines.

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